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FUSED "ONIUM" SALTS AS ACIDS. I. REACTIONS IN FUSED AMMONIUM NITRATE

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A study by Franklin, Kraus and their co-workers of many different reactions[1] in liquid ammonia as solvent has served to establish definitely the existence of a series of ammono-compounds which are derived from ammonia, as the parent substance, in much the same way that the usual acids, bases and salts are related to water. Other investigators have shown that this behavior is by no means limited only to water and ammonia as solvents. Many other substances, such as hydrazine, hydrogen sulphide, hydroxylamine, acetic acid, phosgene and sulphur dioxide may serve as parent solvents for solvo-systems[2] of acids, bases and salts.

General recognition of these facts has resulted in a decided change in opinion as to the definitions of certain classes of compounds, particularly when it was shown that many so-called salts, when dissolved in solvents other than water, exhibited properties typical of acids. Further than this, the acid or basic character of many substances, as defined by their behavior in water, was found to change completely upon solution in other solvents. Thus, urea reacts with the ammono-base, sodium amide, in liquid ammonia to yield a well-defined salt, but in 100 per cent acetic acid it may be titrated with perchloric acid. Such apparent anomalies made it necessary to characterize an acid or a base specifically in relation to a particular solvent. Considerable confusion resulted, especially with respect to nomenclature, since the number of solvent systems could apparently be increased without limit.

It was Brönsted[3] who brought order in a field which threatened to become chaotic when he demonstrated that the phenomenon of acidity was largely a matter of competition between the solvent, and the acid anion for the proton. Acidity in basic solvents, that is, in those media which are characterized by their high affinity for the proton, is due primarily to formation of the corresponding "onium" ion. Thus, hydrogen chloride becomes an acid in liquid ammonia, because the solvent has a greater

affinity for the proton, than the very weak base, the chloride ion. In the equation

$$A_1 + B_2 \rightleftharpoons A_2 + B_1$$
$$HCl + NH_3 \rightleftharpoons NH_4^+ + Cl^-$$

the equilibrium is shifted far to the right.

Actually, it has been found that ammonium salts when dissolved in liquid ammonia react with metals to liberate hydrogen, with amides and nitrides to give salts.[1] Hydrazine salts in anhydrous hydrazine[4] also react chemically as acids. It therefore seems highly probable that all "onium" salts, when dissolved in the respective anhydrobases as solvents, will give reactions typical of acids. It should be pointed out, however, that this statement awaits more extended experimental verification.

The question naturally arises, Do "onium" salts, which have been characterized as acids in their respective parent solvents, exhibit these properties by themselves when undergoing reactions in the dry or fused state? Does the presence of the "onium" ion give to compounds, ordinarily termed salts, the chemical properties of an acid? The answer is most decidedly in the affirmative. The characterization of "onium" salts as acids, *per se*, in the dry and fused state is one of the most useful extensions of the modern Brönsted theory of acidity. Indeed, the very simplicity of this concept has caused it to escape investigation until now.

The authors have studied a whole variety of "onium" salts (ammonium, hydrazine, hydroxylamine, pyridine, aniline, phenylhydrazine compounds) from a purely chemical point of view and find, without exception, that these act as acids in the dry and the fused state. The literature contains countless examples of reactions, involving the use of compounds of this type, which may be interpreted from this point of view. Among these may be mentioned: (1) The fluxing action of ammonium sulfate and ammonium fluoride in the opening up of ores; (2) isolated cases of reactions of metals, oxides and carbonates with ammonium salts[5]; (3) the preparation of anhydrous chlorides by heating the hydrated chlorides in the presence of ammonium chloride, the latter serving to repress hydrolysis and the formation of basic salts; (4) solvolytic reactions, such as the preparation of guanidine thiocyanate by heating ammonium thiocyanate. All of these may be considered as typical acid reactions from this new point of view.

To bring out this behavior in more detail we are reporting in the present article the chemical behavior of fused ammonium nitrate. Ammonium nitrate is stable at its melting point. It is a good conductor of the electric current and is therefore presumably ionized. Whether ionization is simple or involves association is not pertinent at the moment. Suffice it to say,

that the presence of the "onium" ion, which under circumstances may lose the proton, gives to the fused salt the properties of an acid.

Experimental.—Solubility of oxides and carbonates in fused ammonium nitrate.—Metallic oxides, such as CuO, UO_3, MgO, CdO, PbO, CaO, NiO, HgO, BaO and ZnO dissolve in fused ammonium nitrate with formation of the metallic nitrate, ammonia and water. Considered from the old classical viewpoint as extended by the concept of solvo-systems these reactions are intermediate between *aquo*-neutralization and *ammono*-neutralization. Thus, equation (1) represents the reaction between ammonium nitrate, as an ammono-acid in liquid ammonia, with calcium nitride, a basic anammonide; equation (2) the action of nitric acid upon calcium oxide in water as solvent; equation (3) an example of a reaction in fused ammonium nitrate, in which the latter behaves as an acid.

$$6\ NH_4NO_3 + Ca_3N_2 \longrightarrow 3Ca(NO_3)_2 + 8\ NH_3 \quad (1)$$

$$2HNO_3 + CaO \longrightarrow Ca(NO_3)_2 + H_2O \quad (2)$$

$$2NH_4NO_3\ (\text{fused}) + CaO \longrightarrow Ca(NO_3)_2 + 2NH_3 + H_2O. \quad (3)$$

No reaction is observed between fused ammonium nitrate and BeO, Al_2O_3, Cr_2O_3, Fe_3O_4, Fe_2O_3, ThO_2, SnO_2 and the more acidic oxides Ta_2O_5, Cb_2O_5, V_2O_5, MoO_3 and WO_3.

It is rather a point of interest to note that such nitrates as $Th(NO_3)_4$, $Be(NO_3)_2$ and $Al(NO_3)_3$ are soluble in fused ammonium nitrate, even though the oxides apparently do not react.

Such carbonates, as $BaCO_3$, $MnCO_3$, $CaCO_3$ also react with fused ammonium nitrate to yield the corresponding nitrates.

Fused Ammonium Nitrate as an Oxidizing Agent.—Metals above hydrogen in the electromotive series, such as Mg, Zn, Cd, Co, Ni and Pb react vigorously with fused ammonium nitrate to form the corresponding nitrates nitrogen, ammonia and water. Such metals as copper and bismuth also dissolve. Semi-quantitative experiments indicate that reaction takes place in accordance with the equation:

$$Cu + 3NH_4NO_3 \longrightarrow Cu(NO_3)_2 + N_2 + 2NH_3 + 3H_2O.$$

Aluminum, manganese, chromium, iron and tin as well as the less active noble metals are apparently unaffected (possibly due to the formation of an insoluble oxide film since the corresponding oxides are insoluble).

A fused melt containing a small amount of ammonium chloride dissolves gold. This reaction reminds one of the action of aqua regia upon the noble metals.

The sulphides of zinc and lead are attacked slowly to yield a mixture of the corresponding nitrates and sulphates, some hydrogen sulphide, ammonia and water. Here partial oxidation has taken place. Cuprous oxide is

oxidized to the cupric state to give cupric nitrate, ferrous chloride to ferric oxide, stannous chloride to stannic oxide and chromium nitrate to the dichromate stage. The last reaction is distinctly interesting since the hydrated chromium nitrate dissolves to give a green melt which rapidly changes to the yellow-orange color of the dichromate. Complete oxidation takes place.

Electrolysis of Fused Ammonium Nitrate Melts.—It was hoped that the use of low melting fused salts might make possible the electrodeposition of certain metals, but several trial runs soon made it obvious that these melts were too strongly acidic to allow most metals to exist in contact with them. This is especially true in the case of metals above hydrogen in the electromotive series. In the case of the less active metals deposition was observed to take place while the current was being passed through the melt. Thus, arsenic is deposited from a melt composed of ammonium nitrate and ammonium arsenate, bismuth from a bismuth nitrate-ammonium nitrate melt, antimony from a nitrate melt. In the latter two cases the formation of the deposit takes place during electrolysis but as soon as the current is turned off the metals immediately go into solution.

Dehydration of Nitrates in the Presence of Fused Ammonium Nitrate.—Anhydrous lanthanum nitrate has been prepared by solution of the oxide in fused ammonium nitrate[6] and subsequent decomposition of the excess of solvent at higher temperatures. Booth[7] prepares an anhydrous mixture of beryllium nitrate and ammonium nitrate by dissolving the hydrated nitrate in fused ammonium nitrate. The authors have found that such mixtures can also be prepared, in the case of thorium and manganese, possibly due to the formation of anhydrous double ammonium nitrates. The anhydrous nitrate free from ammonium nitrate can be prepared successfully only in those cases where the nitrate itself is stable at the temperature necessary to decompose the excess solvent. Thus, any attempt to prepare the anhydrous nitrates of thorium, beryllium and manganese by complete decomposition of the excess ammonium nitrate results in the formation of the respective oxides before the excess of ammonium nitrate can be removed.

Acid Catalysis by Means of Fused Ammonium Nitrate.—At least one example of such a reaction can be gleaned from the literature. Here again it is necessary to make use of the concept of ammono-compounds. Franklin[8] has shown that a whole series of ammonia derivatives of carbonic acid may be regarded as ammono-acids. Thus cyanamide, dicyanodiamide, biguanide and guanidine are simple ammono-carbonic acids, behaving in liquid ammonia as acids and yielding well-defined salts. These ammono-compounds can be hydrolyzed to yield carbon dioxide and ammonia, a reaction which is catalyzed by the presence of the hydrogen ion. Dicyanodiamide[9] can be ammonolyzed to guanidine by the action of fused am-

monium nitrate. It is significant in this connection, to point out that pure ammonia, itself, even under pressures does not bring about this reaction with any measurable rapidity. The reaction, employing fused ammonium nitrate, is therefore a case of ammonolysis catalyzed by the hydrogen ion, or actually by the bearer of acidity, the ammonium ion.

$$(CNNH_2)_2 + 2H_2O \xrightarrow{H_3O^+} 2CO_2 + 4NH_3$$

$$(CNNH_2)_2 + 2NH_3 \xrightarrow{NH_4^+} 2C(NH)(NH_2)_2.$$

[1] For a comprehensive treatment of reactions in liquid ammonia see articles by Johnson, W. C., and Fernelius, W. C., *J. Chem. Education*, **5**, 664–70, 828–35 (1928); **6**, 20–25, 441–50 (1929); **7**, 981–99, 1291–9, 1602–16, 1850–8, 2600–8 (1930).

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[3] See articles by Hall, N. F., on modern conceptions of acids, *J. Chem. Education*, **7**, 782–93 (1930); *Chem. Reviews*, **8**, 191–211 (1931).

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[7] Booth, H. S., and Torrey, G. G., *Ibid.*, **52**, 2581–2 (1930).

[8] Franklin, E. C., *Ibid.*, **44**, 486–509 (1922).

[9] Smith, G. B. L., Sabetta, V. J., and Steinbach, O. F., Jr., *Ind. Eng. Chem.*, **23**, 1124–29 (1931).

NORMS OF POLLEN-TUBE GROWTH IN INCOMPATIBLE MATINGS OF SELF-STERILE PLANTS

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A few years ago, fifteen self-sterility allelomorphs of the single gene *S* were found in *Nicotiana Sanderæ* Hort., *N. alata* Lk. and Otto, and their hybrids. Subsequently, these allelomorphs were combined in various ways, including a complete series in union with S_1. All these combinations were then selfed, advantage being taken of the fact that some seed is usually obtained when the flowers are pollinated between 24 and 48 hours before they are due to open. Plants homozygous for all the allelomorphs were obtained by this method, and were again selfed. Data were thus accumulated which throw some light on the norms of pollen-tube growth of the various types under given conditions, and on the effects of disturbed conditions on these norms.

Plants were judged in two ways: (*a*) as to ease of selfing, and (*b*) as to the constitution of the resulting progeny. The first judgment is an esti-

mate of what happens when a series of plants of identical S constitution (say S_1S_1), but differing in other genes affecting flower color, pollen color, anther color, style length and the like, are pollinated under comparable conditions. It is based upon frequency of capsule setting, size of capsule and number and type of seed per capsule. The second judgment is based upon the relative frequency with which plants of definite S constitutions are obtained in matings where there is competition between the pollen tubes. For example, unless there is selective elimination of zygotes, which is known to be significant only in the case of S_3 through close linkage with a semi-lethal gene, then the relative proportion of S_1S_1 and of S_5S_5 plants obtained after selfing an S_1S_5 plant, gives the comparative effectiveness of S_1 and S_5 pollen.

In the course of this work, the particular S allelomorphs involved have been determined in more than ten thousand individuals. At least one hundred thousand selfings have been made. From this considerable experience, the families can be divided into five groups. The classification is necessarily less of a quantitatively objective grouping than is ideally desirable; but it is distinctly more precise than one having no experience with the material would suppose. The scoring was done upon populations having a given S constitution which varied in size from 10 plants to 230 plants, the average being approximately 60 plants. From 1 to 12 populations of each composition were scored.

An example, taken at random, will serve to show the character of the data available. Flowers from each of the 38 plants of an S_1S_8 population were selfed just after the blossoms had opened and the anthers had begun to dehisce. Flowers comparable as to order on the flower stalk, etc., were also selfed in the bud, that is, between 24 and 48 hours before they might be expected to open. Now, in spite of the facts that this population showed segregation for 3 genes affecting flower color, that the plants had styles varying from 38 mm. to 58 mm., that some plants were more vigorous than others and that the average "life" of the pollinated flowers varied from 3 to 6 days, the plants reacted to selfing in a manner extraordinarily uniform. From 156 normal selfings, only 2 small capsules were obtained (upon different plants), each containing about one-tenth the normal complement of seed. From 105 bud selfings, all but one plant—and it was pollinated only twice—yielded capsules averaging about one-half their full complement of seed. Every pollination was not successful, as would obviously be true in any type of pollination test; but 64 capsules were obtained having a sharp mode at the one-half complement point. Extensive tests of this character gave the following classification.

Group I.—Plants of Group I produce small capsules at all times during the flowering season when the open flower is pollinated. Genotypes belonging to this group are S_1S_{15}, S_8S_{15} and $S_{15}S_{15}$.

Group II.—Plants of Group II frequently produce small capsules when the open flower is pollinated, but only near the end of the flowering season. Genotypes belonging to this group are S_1S_2, S_1S_9, S_2S_2, S_9S_9, (S_2S_4), (S_2S_5) and (S_2S_7). *N. B.* The genotypes placed in parentheses in a given group are cases where a single family behaved as if it belonged in that group. The same genotype will be found in another group without parentheses, since the reactions of the majority of the families placed them in that group.

Group III.—Plants of Group III produce capsules but rarely when the open flower is pollinated; but they regularly produced capsules which average half of the full complement of seed when bud pollinations are made. Genotypes belonging to this group are (S_1S_2), S_1S_3, S_1S_6, (S_1S_7), (S_1S_8), S_1S_{10}, S_1S_{11}, S_1S_{12}, S_2S_3, S_2S_4, S_2S_5, S_2S_6, S_2S_9, S_4S_{13}, S_7S_{10}, S_9S_{10}, $S_{11}S_{12}$, (S_2S_2), S_3S_3, S_6S_6, S_7S_7, S_8S_8, (S_9S_9), $S_{11}S_{11}$, $S_{12}S_{12}$ and $S_{13}S_{13}$.

Group IV.—Plants of Group IV show no pseudo-fertility under ordinary conditions when the open flower is pollinated, but often yield small capsules when bud pollinations are made. Whereas bud pollinations in Group III nearly always result in capsules containing between 40 and 70 per cent of the full complement of seed, bud pollinations in Group IV result in approximately a 25 per cent set, and the capsules seldom contain over 20 per cent of the full complement of seed. Genotypes belonging to Group IV are (S_1S_3), (S_1S_5), S_1S_7, S_1S_9, S_1S_{13}, S_1S_{14}, S_3S_6, S_5S_6, S_5S_{14}, S_7S_8, S_8S_{11}, (S_1S_1), S_7S_7, S_8S_8, $S_{10}S_{10}$ and $S_{14}S_{14}$.

Group V.—Plants of Group V show no pseudo-fertility, and very rarely produce a small capsule after a bud pollination. Genotypes belonging to Group V are S_1S_4, S_1S_5, S_1S_1, S_4S_4 and S_5S_5. Two families out of nine families of S_1S_5 investigated yielded a few small capsules after bud pollinations. One family of S_1S_1 belonged in Group IV; eleven similar families belonged in Group V. No seeds were ever obtained from selfing S_1S_4, S_4S_4 or S_5S_5. The S_4S_4 plants and the S_5S_5 plants produced were obtained by selfing S_2S_4 and S_2S_5 plants.

A consideration of these facts, together with certain data on the conditions under which the selfings were made, appears to warrant the following conclusions.

In plants homozygous for the various S factors, S_1S_1–$S_{15}S_{15}$, each allelomorph exhibits a specific norm for pollen-tube growth. The spread between the growth rates for the fast-growing tubes of $S_{15}S_{15}$ and the slow-growing tubes of S_4S_4 and of S_5S_5 is such that the one must grow approximately twice as fast as the others. This conclusion is based upon estimates of time of fertilization. The growth-rate norm is influenced but slightly by the type of gene carried in other chromosomes than that carrying S, as evidenced by the similar reactions of plants in families segregating for 4, 5 or 6 color genes, and for genes producing style length differences of

30 mm. The influence of temperature differences between 20°C. and 30°C. is not detectable. Within the limits of normality, the physical condition of the plant, the relative length of daylight and darkness or the period in the life-cycle when the pollinations are made, have no detectable effect on the growth rate, except that during the last week of the flowering season the effective fertility in incompatible matings is materially increased. To a minor extent this increased fertility is due to a measurable increase in the "life" of the flower after pollination, averaging about 24 hours. The major factor influencing the rise in fertility, however, is a stepping up of the pollen-tube growth rate of each of the various S genes that is approximately the same in every case.

The establishment of specific norms for pollen-tube growth in the various S allelomorphs would seem, at first sight, to indicate a simple quantitative relationship among the genes involved. One can imagine a series of mutations at the S locus which cause an increase (or decrease) in the capacity of the pollen grain or of the style for producing the substances required for pollen-tube growth, even though some of the allelomorphs have norms so similar that they cannot be separately identified by the method of experimentation used. But it seems to me that the mechanism which is at the basis of this process is more complicated than this. Unquestionably, a simple nutritional chemistry is implicated. But the essential distinctions between these allelomorphs depend not on nutritional chemistry, but rather upon some more complicated type of chemical reaction between the pollen tube and the style, which closely resembles the specific proteid reactions belonging to immunological phenomena. The principal evidence in favor of this conclusion is that, though S_7S_7, S_8S_8, $S_{10}S_{10}$ and $S_{14}S_{14}$ plants have similar growth rates in individual self-pollinations, each is compatible with the other in cross-matings. In the cross-matings, the pollen-tube growth rates are tremendously accelerated. This hypothesis, moreover, is not unreasonable, since the pollen tube is virtually a parasitic organism growing within the style.

Finally, attention must be called to the noticeable effect upon pollen-tube growth which the presence of one S allelomorph has upon another. A normally slow-growing allelomorph is speeded up by the presence of a fast-growing allelomorph in matings where both are incompatible. For example, S_1S_1, S_4S_4 and S_5S_5, all belong to Group V, which includes only plants from which it is practically impossible to obtain seeds after bud selfings. S_1S_4 plants and S_1S_5 plants belong to the same group. Yet when these allelomorphs are combined with S_2, which, in the homozygous form, belongs in Group II, S_1, S_4 and S_5 pollen-tube growth rates are stepped up so that they fall into Group III. Such reactions are detectable, however, only when the competition is between two types of incompatible pollen tubes. When pollen from S_1S_4 or S_1S_5 plants is placed upon the stigma

of a mature flower of an S_1S_1 plant, the resulting progeny are all S_1S_4 plants or S_1S_5 plants, as the case may be. No S_1 pollen functions.

Naturally, it will be asked if greater ease of obtaining seeds from bud selfings of S_1S_2, S_2S_4 and S_2S_5 plants is not due merely to the effectiveness of the fast-growing S_2 pollen tubes belonging to Group II. That this is not the case is shown by the frequency with which plants of different constitutions are obtained. A fairly large amount of data is available, but a few examples will serve to show the character of the facts. When pollen tubes of the same general growth-rate norm are in competition, the results accord with Mendelian expectation both in compatible and in incompatible matings. When S_1S_1 plants are pollinated with S_7S_8 pollen, S_1S_7 and S_1S_8 plants are obtained in essentially equal numbers. Similar proportions of the homozygotes, S_7S_7 and S_8S_8, are obtained when S_7S_8 plants are selfed in the bud. On the other hand, when plants like S_1S_2 or S_2S_4 are selfed in the bud, the proportions of the two types of homozygotes in the resulting population are unlike. One set of three families obtained from S_1S_2 plants selfed in the bud yielded 29 S_1S_1 plants and 78 S_2S_2 plants, together with 155 S_1S_2 plants. Two families arising from S_2S_4 plants selfed in the bud included, besides the expected S_2S_4 plants, 56 S_2S_2 plants and 16 S_4S_4 plants. Since S_1S_1 plants and S_4S_4 plants selfed in the bud have been found to be almost completely infertile, it is evident that the presence of S_2 pollen tubes had an accelerating effect on their more slowly growing sisters. But that each allelomorph tended to retain its own growth norm is shown by the difference in the proportion of homozygotes obtained.

Whether there is a similar acceleration of incompatible pollen tubes when in competition with compatible pollen tubes, is not known. Such might well be the case; but, if so, it is not detectable because of the great differences in rate of pollen-tube growth in the two types. The only information available is that the compatible pollen tubes are always effective, while the incompatible pollen tubes are not.

It seems likely that the immediate cause of the acceleration effect is an interference with the specific incompatibility reaction rather than with the general nutritive reaction. The nutritive reaction appears to be comparatively simple if one may judge from studies of pollen-tube growth on artificial media and from the fact that growth is often normal in intergeneric matings. If one assumes that the nutritive reaction is concerned largely with the availability of soluble carbohydrates and salts which can readily pass outside the membranes of the cells of the style, then an excess of such nutrients ought sometimes to be formed and to be available to the more slowly growing pollen tubes. In this case, if a stigma were pollinated with a small amount of incompatible pollen and a large amount of compatible pollen, or if a small amount of S_4 pollen and a large amount of S_2 pollen were placed on an S_2S_4 stigma, then a few incompatible unions should be

effective in the first experiment, and an unusually large number of S_4S_4 plants should be obtained in the second experiment. Both of these experiments have been tried, and the results have been negative. It seems, therefore, that the major effect of these associations between slow-growing and fast-growing pollen tubes is upon the compatibility-incompatibility reactions which form the second important feature in the biology of pollen-tube growth.

A NEW GENE AFFECTING BEHAVIOR AND SKELETON IN THE HOUSE MOUSE

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A new recessive mutant character has recently appeared in the house mouse which is unusual in combining effects similar to those associated with two previously known genes. The new mutation is known as shaker-short (symbol s^f). It is recognizable at birth by the shortened tail which varies from no bony tail at all (one case) to three-fourths of the length of the normal tail and usually ends in a slender filament which contains no vertebrae and falls off soon after birth. It resembles closely the brachyury mutant described by Dobrovolskaia-Zavadskaia and Kobozieff (1927). Brachyury, however, acts as a dominant and is lethal when homozygous.

Beginning at about five days of age, the new mutant type shows severe disturbances in equilibration. Erratic circus movements, more chaotic than those seen in the waltzing mouse, appear a little later, and it shows an ataxia of the head, accompanied by tremors, more extreme than the somewhat similar condition in the shaker mutations previously described by Dobrovolskaia-Zavadskaia (1928), and by Lord and Gates (1929). As adults the mutants show marked lack of coördination. Most of them show a tendency to circle but seldom consistently in one direction. Generally they appear too weak and erratic for active waltzing. All short-tail mutants which have lived to three weeks of age have developed this extreme choreic behavior. All such have also proved to be completely deaf, this being another point of resemblance to the waltzers and the two different shaker varieties. The fertility of eight adult mutants (four males and four females) has been tested by matings with normal mice. All have been completely sterile.

In addition to these peculiarities many of the mutants have at birth a marked lesion near the median point of the parieto-occipital suture. The

lesion appears either as a bleb filled with blood or lymph which later dries to a small scab, or it may consist of such a scab at birth. This may provide a valuable clue to the central nervous disturbances which appear later.

The new type acts as a simple recessive to normal. Since the homozygotes are sterile, the only data are those from matings of heterozygotes. To date such matings have produced 120 offspring of which 85 were normal and 35 were short-tailed at birth, the monohybrid expectation being 90:30. Only litters from known heterozygotes are included and since the production of one mutant offspring is required to establish the heterozygosity of the parents, it is probable that there have been omitted several litters, consisting of normals only, from heterozygous parents which have not yet produced a mutant. For this reason the segregation ratio cannot be used for judging the prenatal viability of the mutant. 29 litters borne by heterozygotes mated *inter se* have given an average of 4.1 young per litter; 35 litters from the same heterozygotes mated to homozygous normal sibs have given 4.9 young per litter, so there may be a little higher mortality among the mutants before birth.

The new mutation appeared in a stock unrelated to any of the mutant types which it resembles. The stock originated from a pair of hairless mice caught wild in London in 1924 and bred by Mr. H. C. Brooke who sent several specimens to Professor F. A. E. Crew of Edinburgh University in 1925. Professor Crew was kind enough to give me, in 1927, a male and two females, heterozygous for the hairless gene (as well as for piebald, indicating that outcrossing had occurred) and these were inbred for the hairless character, with the exception of one outcross to the Bagg albino stock ten generations before the mutant appeared. Because of the striking nature of the new variant and the antecedent inbreeding we may assume that the new complex of characters arose by mutation within the last few generations.

This is the fourth recorded mutation in mice affecting equilibration and nervous coördination in similar ways. The previous three, viz., waltzer, shaker 1 (Lord and Gates), shaker 2 (Dobrovolskaia-Zavadskaia) have all been shown to be due to mutations at different loci. The new form, judging by its extremely abnormal behavior and the tail defect associated with it, is probably also due to a separate mutation. It is being tested for allelomorphism with the other mutants, but since the homozygotes are sterile these tests will require some time. In spite of its close resemblance to the brachyury mutant, the fact that it is entirely recessive, not lethal and also choreic indicates that it is probably not a recurrence of this mutation.

The new mutation probably affects the development of both the vertebral column and the brain. A similar effect on the axial skeleton, brought about by the dominant brachyury mutation, has been shown by Chesley

(1932) to involve abnormalities in the early mesoderm and neural tube. One step in the determination of the extreme defects in the nervous system of the homozygous form is the failure of the notochord to differentiate (Chesley[1]). Genes which affect such fundamental tissues are of prime importance in attempting to relate the gene to the processes of differentiation, and the new material appears to be especially valuable for this purpose. Since previous attempts to disclose the structural defects responsible for the waltzer and shaker conditions have not been successful, an investigation of the early development of the brain in the new form should be of unusual interest.

[1] Dissertation (unpublished).

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PROJECTIVE DIFFERENTIATION OF SPINORS

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In the paper by Veblen and Taub on "Projective Differentiation of Spinors" in this volume of these Proceedings, **20**, 85–92(1934), it is shown that $\gamma_{,\lambda} = \gamma f_\lambda$ where[1]

$$f_\lambda = \frac{1}{2} \text{ Trace } \left(\gamma^{-1} \frac{\partial \gamma}{\partial x^\lambda} \right)$$

is a necessary condition for the reality of $\Gamma^\alpha_{\beta\lambda}$. It is then stated that: "Hence the conditions (2.1), (2.2), (2.3) and (2.4) are self consistent and imply that $A_{\beta\lambda\mu}$ is real and anti-symmetric in β and μ but otherwise arbitrary." Since (2.1) states that $\gamma_{,\lambda} = 0$ this conclusion does not follow without further argument, but fortunately the missing step is not hard to supply.

Writing the fifth equation of paragraph seven in the form

$$K^*_\lambda = \gamma^{-1} K_\lambda \gamma + \gamma^{-1} \frac{\partial \gamma}{\partial x^\lambda} - \frac{1}{2} \text{ Trace } \left(\gamma^{-1} \frac{\partial \gamma}{\partial x^\lambda} \right) .1$$

and taking the trace gives

$$\text{Trace } \left(\gamma^{-1} \frac{\partial \gamma}{\partial x^\lambda} \right) = 0.$$

Hence $f_\lambda = 0$ and a necessary condition for the reality of $\Gamma^\alpha_{\beta\lambda}$ is $\gamma_{,\lambda} = 0$. By (7.1) it is sufficient. This completes the proof of the last statement in the Veblen and Taub paper. An independent verification of the vanishing of f_λ follows from differentiating equation (4.6) of these PROCEEDINGS, **19,** p. 982 (1933), or from the invariant normalization

$$\text{determinant of } \gamma = 1$$

which is made in (7.7), p. 985.

[1] The expression given by Veblen and Taub for f_λ (p. 90) contains a misprint which is corrected by our formula. Other corrections are:

p. 86, line 5, for $\psi^C * \Delta^B_\lambda$ read $\psi^C * \Delta^B_{\dot{C}\lambda}$;

p. 86, line 21, for $\gamma_{AB}\, \psi_{,\alpha}^{\beta}$ read $\gamma_{AB}\, \psi_{,\lambda}^{B}$;

p. 87, for right member of last equation read $\frac{1}{2}\left(\delta^A_G\, \delta^E_B + \gamma^{AE}\, \gamma_{BG}\right)$;

p. 88, the right member of (4.6) should contain the additional term

$$-\frac{1}{4}\ \text{Trace}\left(\gamma_\alpha \frac{\partial \gamma^\alpha}{\partial x^\lambda}\right).1;$$

p. 89, equation (6.2) and next equation, for $\gamma^{AE}\gamma_{BC}$ read $\gamma^{AE}\gamma_{CB}$;

p. 90, line 2, read $= \frac{1}{2}\gamma_D^{C\alpha}\ (\gamma^{AD}\gamma_{CB})_{,\lambda}$;

p. 90, line 11, delete "It is not a necessary condition";

p. 90, line 12, the right member of the equation should contain the additional term

$$-\frac{1}{4}\ \text{Trace}\left(\gamma_\alpha \frac{\partial \gamma^\alpha}{\partial x^\lambda}\right)^*.1;$$

p. 91, line 13, for $x^0, x^1, \ldots, x^4$ read $X^0, X^1, \ldots, X^4$;

p. 91, lines 17 and 18, read $X^0 = e^{x^0}$, $X^i = e^{x^0}\, x^i$ $(i = 1, \ldots, 4)$;

p. 91, last line, for $\Gamma^c_{c\lambda}$ read $\Gamma^C_{\dot{C}\lambda}$.

Professor Veblen asks me to note the following corrections to his "Spinors in Projective Relativity," these PROCEEDINGS, **19,** 979–989 (1933):

p. 985, line following (7.9), for $\gamma_{\alpha\beta}$ read γ_{AB};

p. 987, equations (9.4), for $\gamma_{A\dot{B}}\psi^A\psi^{\dot{B}}$ read $\gamma_{A\dot{B}}\psi^A\psi^{B*}$;

p. 987, equations (10.3), for $S^{\alpha\beta}_{A\dot{B}} = S^{\alpha\beta\,*}_{B\dot{A}}$ read $S^{\alpha\beta}_{A\dot{B}} = -\,S^{\alpha\beta\,*}_{B\dot{A}}$.

THE EXTINCTION OF CHAINED REFLEXES

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Communicated March 13, 1934

The extinction of the conditioned initial member of a chain of reflexes may be brought about by interrupting the chain at any point prior to the unconditioned reflex upon which the conditioning is based. Consider the following example:

$$S^{III} - R^{III} \xrightarrow{(A)} S^{II}_D - R^{II} \xrightarrow{(B)} S^{I} - R^{I}.$$

Let $S^{I} - R^{I}$ be a reflex (capable of further analysis) in which the response R^{I} is the seizing, chewing and swallowing of food and in which the stimulus S^{I} is composed of tactual and olfactory stimulation arising from the food. Let R^{II} be the approach to the food tray and S^{II} the stimulation arising from the tray and adjoining parts of the apparatus plus a differentiating component (D) supplied by the sound of a food magazine. Let R^{III} be the pressing downward of a lever and S^{III} the stimulation arising from the lever and adjoining parts of the apparatus. For our present purposes $S^{I} - R^{I}$ may be considered an unconditioned reflex. The second link is established as the conditioned reflex $S^{II}_0 - R^{II}$ by chaining R^{II} to S^{I}.[1] A discrimination between S^{II}_D and S^{II}_0 is then established, the response to S^{II}_0 being extinguished.[2] The initial link $S^{III} - R^{III}$ is then conditioned by chaining R^{III} to S^{II}_D or, more exactly, to the differentiating member D. Then it is possible to extinguish the reflex in response to the lever ($S^{III} - R^{III}$) by breaking the chain either at the juncture $R^{III} \longrightarrow S^{II}_D$ (A) or at $R^{II} \longrightarrow S^{I}$ (B). We may consider the following cases:

I. Breaking the chain at A we obtain the simple extinction of $S^{III} - R^{III}$, since we establish the essential condition by removing the reinforcing stimulus S^{II}_D.[3] Our method does not supply information about $S^{II}_D - R^{II}$ directly, since it measures only the rate of responding to the lever, but it is clear from inspection that $S^{II}_D - R^{II}$ remains fully conditioned. After deep extinction of $S^{III} - R^{III}$ the sound of the magazine (completing S^{II}_D) will evoke R^{II} immediately.

II. If we reëstablish the connection at A, the first response to S^{III} reconditions the reflex, and if we then break the connection again, we obtain another extinction curve (page 306 in the second reference).

III. If, now, we reconnect at A but break at B, we also get reconditioning of $S^{III} - R^{III}$, because the presence of S^{II}_D is an adequate reinforcement, whether or not the later stages of the chain are reinforced (page 427 in reference 5). But as $S^{II}_D - R^{II}$ will now be extinguished, it will not continue to reinforce $S^{III} - R^{III}$, and the latter reflex will decline in strength.

The extinction of the first member of the chain due to the failure to reinforce the second has been described.[3] The effect of reconnection at A is shown typically in figure 1A. The curve is a record made automatically by a white rat as previously described.[3] The first part of the curve shows the extinction of $S^{III} - R^{III}$ when the chain is broken at A. A characteristic cyclic deviation is evident. At the arrow the connection at A was restored (by connecting the magazine) but that at B had been broken (by leaving the magazine empty). It will be seen that the response to the lever is quickly reconditioned, and then goes through a second decline corresponding to the extinction of the reinforcing reflex $S_D^{II} - R^{II}$. More

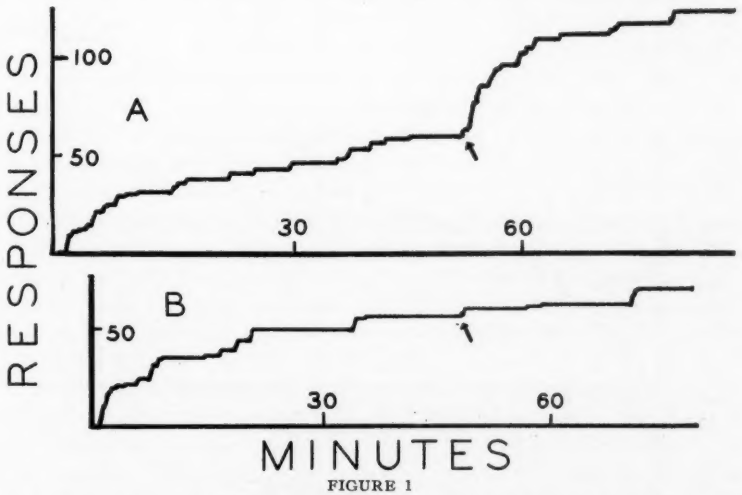

FIGURE 1

A. Extinction curve where the chain is broken at A (see diagram in text). At the arrow the chain is reconnected at A but broken at B. Another extinction curve follows.

B. Extinction curve where the chain is broken at B. At the arrow the chain is broken at A, but there is no significant effect.

regular curves, of a slightly different shape, may be obtained by using extinction after periodic reconditioning,[2] examples of which are given in figure 2. The figures A and B correspond to figure 1A. They show the effect of different initial strengths of the reflex, which give different slopes to the curves.

IV. If we break the chain at B without having broken at A, $S^{III} - R^{III}$ continues to be reinforced by S_D^{II} but declines in strength with $S_D^{II} - R^{II}$ as in Case III. This is shown in the first parts of figures 1B and 2C.

V. If we reconnect at B for one response, a subsequent extinction curve is obtained as in III.[3]

VI. If we break at A after having broken at B we get no new extinction curve for $S^{III} - R^{III}$ alone. This is shown typically in figures 1B and 2C.

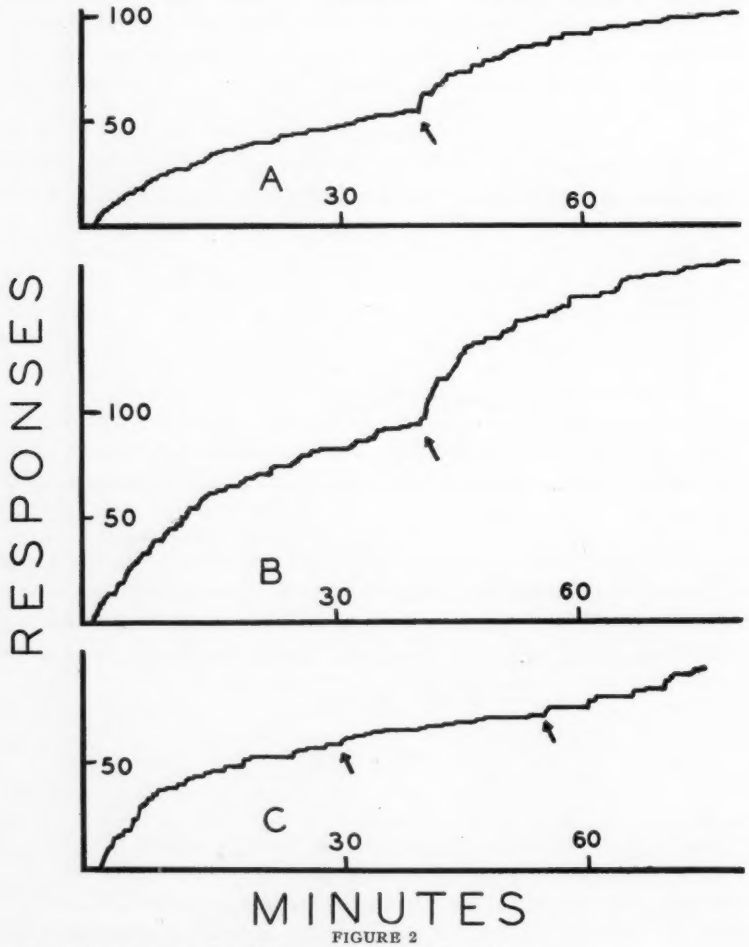

FIGURE 2

Extinction curves after periodic reconditioning.

A and B. Chain broken first at A; then (at the arrows) at B.

C. Chain broken first at B; then (at the first arrow) at A. At the second arrow the connection at A is restored. No significant effect of either change is observed.

The first part of each curve is for extinction at B. At the arrow the chain was broken at A. The second arrow in figure 2 marks the restoration of the chaining at A and shows no significant effect.

We may set up this rule: that the interruption of a chain extinguishes all members up to the point of interruption but not beyond. Since the interruption suppresses the elicitation of all members coming after it, the rule may be stated more significantly as follows: *in a chain of reflexes not ultimately reinforced only the members actually elicited undergo extinction.*

The rule does not state whether the elicited members decline in strength simultaneously or not. We have implied in III that they do not. It is a simpler assumption that the interruption does not have a backward effect upon the whole chain. With the present method it is difficult to show this because we examine only the initial member. It would require many more observations than are now available to settle the point by comparing curves for simple (break at A) and remote (break at B) extinction, since, as we have seen, there are considerable individual variations in the N_e/N_c ratio,[2] even when the degree of conditioning has been controlled. (The first curves in figure 1 are approximately of the same area only because we have selected these examples to aid the comparison.) Curves for original extinction are also unfortunately marked by local deviations. It has been pointed out elsewhere on the information then available that there seems to be "no significant difference in the rate or the change of rate" between the two cases.[3] Nevertheless it is probable that, in a long chain at least, we could obtain a greater total number of elicitations of the initial member by breaking successively at A, B, C, . . . N than by breaking immediately at N, and that the curve in the latter case would differ from the curve at A in the former.

The question is important. We are justified in regarding a continuous and integrated act of this sort as a chain, not only because the links of which it is composed are put together one by one, but because they may be taken apart at will. But the degree of autonomy of a given part cannot be established until the nature of remote extinction is known.

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[1] "On the Rate of Formation of a Conditioned Reflex," *J. Gen. Psychol.*, **7**, 274,286 (1933).

[2] "The Rate of Establishment of a Discrimination," *Ibid.*, **9**, 302–350 (1933).

[3] "On the Rate of Extinction of a Conditioned Reflex," *Ibid.*, **8**, 114–129 (1933).

[4] "Drive and Reflex Strength," *Ibid.*, **6**, 22–37 (1932).

[5] " 'Resistance to Extinction' in the Process of Conditioning," *Ibid.*, **9**, 420–429 (1933).

ANOMALIES IN THE ABSORPTION SPECTRUM OF VISUAL PURPLE

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Since the discovery of visual purple by Boll,[1] and its preparation in solution by Kühne,[2] this bluish red pigment of the vertebrate retina has been associated with the visual process. In the retina and in solution, the color of visual purple disappears upon exposure to light, but the precise changes which the color undergoes in the process have been subject to much controversy. The exact color is of importance in defining the possible chemical change which follows or accompanies the primary photochemical action.

Kühne first observed a distinct yellow stage during the bleaching of visual purple by light. This was confirmed by others, especially by Garten.[3] The yellow color completely disappears on prolonged illumination only with great difficulty. Comparisons of partially bleached solutions with various mixtures and dilutions of bleached and unbleached visual purple solutions, as well as observations of the mechanical thinning of whole retinas, convinced these observers that the intermediate yellow color does not result merely from the dilution of the original substance, but that the yellow color can be produced only by the photic decomposition of visual purple.

The appearance and disappearance of the yellow color was taken by Kühne to indicate that visual purple does not bleach directly to a colorless substance, but that an intermediate stage is involved representing the origin of a new substance during the exposure to light. Kühne called this visual yellow, and Garten even proposed certain chemical steps in the formation of visual yellow from visual purple.

In contrast to these findings are the results of Köttgen and Abelsdorff,[4] who measured the absorption spectrum of visual purple after successive stages of bleaching by light and found no new substance appearing during the bleaching, but instead only the regular disappearance of the absorption band of the original visual purple. Similar results were later reported by Trendelenburg.[5] Furthermore, the regularity of the course of the bleaching of visual purple as measured colorimetrically by one of us[6] indicates the absence of any intermediate substance, because the color of the solution in the course of bleaching may be adequately matched with mixtures of bleached and unbleached solutions.

The most puzzling thing about these two sets of conflicting data has been the apparent validity of both. In fact, in the course of several years,

we ourselves have secured both types of evidence. The original measurements[6] on the kinetics of visual purple bleaching were made in the spring and summer of 1920. They showed no irregularities which could be attributed to the formation of an intermediate substance. Repetition of these colorimetric measurements by one of us (S. H.) in the winter of 1924 distinctly showed such irregularities, since the color of visual purple dur-

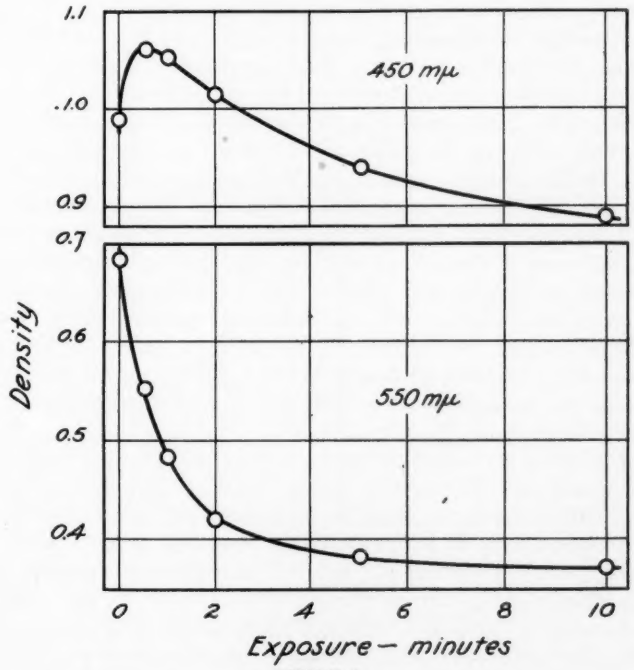

FIGURE 1

The change in density (log I_0/I_t) during bleaching by white light of a solution of visual purple from winter frogs measured at two wave-lengths. At 550 mμ the density decreases steadily during bleaching, whereas at 450 mμ the density first increases and then decreases, thus indicating the transitory formation of a blue-absorbing substance during bleaching.

ing bleaching could not then be properly matched by mixtures of bleached and unbleached solutions.

A study made at that time of the absorption of visual purple in various parts of the spectrum during bleaching gave the reasons for these irregularities. Figure 1 shows the data of one of many similar sets of measurements recording the density of solutions of visual purple during bleaching by

light. Density equals log (I_0/I_t) and is the negative logarithm of the transmitted light fraction. Measured with light of 550 mμ, the absorption of visual purple decreased steadily during bleaching. Measured with light of 450 mμ, the absorption during the same bleaching first rose and then fell, thus clearly indicating the formation of an intermediate substance yellow in color.

Recently Hosoya and Bayerl[7] measured the absorption spectrum of visual purple in various solvents and attributed the two different types of bleaching to differences in the solvent. Later work by Hosoya[8] himself has shown that these differences in the effects of the solvent are not as large as first thought (personal communication), and were probably due to the inherent difficulties of accurately measuring the very small light transmission in the critical blue region of the spectrum. The differences introduced by the solvent, though probably present, are only a minor source of variation. Kühne had found that retinas, hardened in alum before extraction with bile salts, gave solutions which bleached directly to complete colorlessness; and Köttgen and Abelsdorff had used this procedure in some of their preparations. Moreover, Kühne[9] showed that visual purple, precipitated from solution by saturation with $MgSO_4$ and then redissolved, also bleached directly to complete colorlessness. However these procedures of purification have been criticized by Garten as possibly changing the structure of the visual purple itself, and the validity of results themselves have even been disputed by him.

These puzzling contradictions have now been at least partly resolved by measurements made by one of us (A. M. C.) in the last eighteen months. In July, 1932, a solution of visual purple in purified bile salts was obtained which showed almost no indication of the formation of any intermediate substance during bleaching by light. The measurements were made visually with a Koenig-Martens spectrophotometer, and the data resembled those found by Köttgen and Abelsdorff. The solution which gave this result was obtained from freshly collected frogs, and represented an unusually large number of retinas dissolved in a relatively small volume of bile salts solution. The right half of figure 2 gives the data. It shows the density of the solution in the short-wave end of the spectrum at different stages from the unbleached solution (*a*) to the nearly completely bleached solution (*d*). It is apparent that the density decreases regularly at all wave-lengths.

During March of 1933, a solution of visual purple was prepared by a procedure which was exactly similar to that of the previous summer in all but one respect; namely, that the frogs had not been freshly collected but had been kept in cold storage for several months. In the bleaching of this extraction the intermediate yellow color was very pronounced. The data are given in the left half of figure 2 which shows the density at different

wave-lengths for different stages of bleaching. It is apparent that, whereas at wave-lengths greater than 460 mμ the density steadily decreases, at wave-lengths less than 460 mμ the density first rises, and only later begins to decrease, thus corroborating the findings of figure 1.

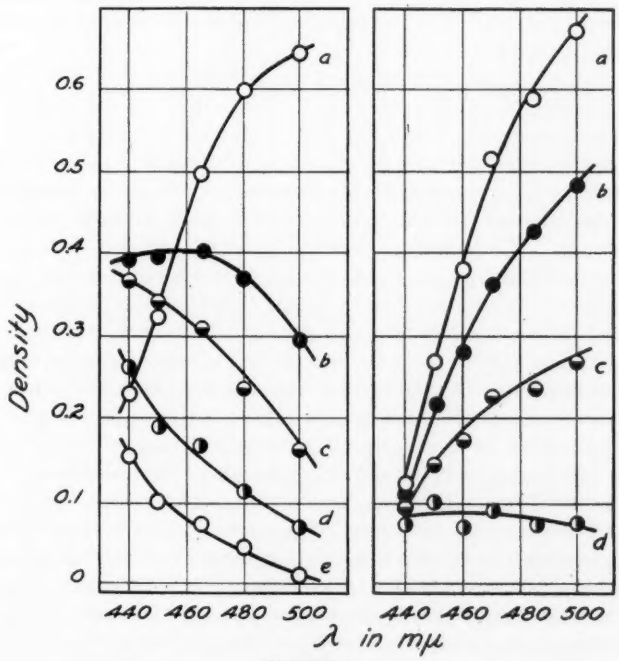

FIGURE 2

The absorption spectrum (short wave-lengths only) of visual purple at different stages (*a* to *e*) of bleaching with white light. The left half records the changes in density of a solution from winter frogs showing clearly the formation of an intermediate substance having strong absorption in the blue. The right half records the changes in density of a solution from summer frogs showing the absence of the blue-absorbing substance during bleaching. The density measurements in the figure record the density of the solution against air minus the density of the solution after prolonged bleaching with intense white light, and thus represent the behavior of the photosensitive materials only and not of the slightly colored but photostable substances also extracted from the retina by bile salts.

In May, 1933, another visual purple extraction was made from the retinas of frogs that after collection had been active in the laboratory for several weeks. In the bleaching of this solution by light there was only a trace

of the formation of an intermediate substance. Extractions of visual purple, prepared during the summer of 1933, in all respects duplicated the results of the previous summer. During bleaching they showed almost no trace of any rise in absorption when measured in the blue end of the spectrum. Since then extractions made during the present winter have shown the usual formation of an intermediate yellow substance in the course of bleaching with light.

Clearly under some circumstances the intermediate yellow color appears in measurably large quantities, while under other circumstances it can barely be observed. In our own experience, retinal extracts from spring and summer frogs show only traces of this intermediate yellow color whereas hibernating winter frogs show it in profusion. Hosoya's recent work in which the yellow color was apparent was also done, as explicitly stated, on winter frogs. We have here then one more example of the well-known, strikingly different, physiological results given by summer and by winter frogs which on several previous occasions have led investigators astray.

Since it is very unlikely that the visual purple itself or that the course of its bleaching are different in the two cases, we must attribute these differences to the presence in varying amounts of some other substance in the extractions. There are several possibilities for such substances. They may be nearly colorless but also light sensitive and form the yellow substance during photic decomposition, or they may have an absorption spectrum like that of visual purple and go through the yellow stage in their photic decomposition. Or, what seems more likely, they may be colored or colorless substances with which the bleached decomposition products of visual purple merely combine to give the yellow substance. The commonly found visual purple is always present, while the other substances are present or not depending in some way on the physiological condition of the frog. The purification of visual purple by the methods of Kühne then consists of either rendering these other substances insoluble or difficultly soluble as with the alum pre-treatment, or of differentially removing visual purple from them by precipitation with saturated $MgSO_4$. In our measurements this is accomplished by the summer and winter conditions of the retina itself.

It is not impossible that these other substances may be significant for the physiology of the retina since the existence in it of absorbing substances like vitamin A[10] has been established and the influence of this substance on the retinal regeneration of visual purple indicated.[11] Further work on the absorption spectrum and other properties of the precursor for the yellow-appearing, intermediate, blue-absorbing substance, and its influence on the reaction kinetics of visual purple is now in progress.

[1] Boll, F., *Monatsber. Berl. Akad.*, 783 (1876).

[2] Kühne, W., *Hermann's Handbuch der Physiologie*, **3**, 235 (1879).

[3] Garten, S., *Arch. f. Ophthal.*, **63,** 1 (1906).

[4] Köttgen, E., and Abelsdorff, G., *Z. Psychol. u. Physiol. der Sinnesorgane*, **12,** 161 (1896).

[5] Trendelenburg, W., *Ibid.*, **37,** 1 (1904).

[6] Hecht, S., *J. Gen. Physiol.*, **3,** 1 (1920).

[7] Hosoya, Y., and Bayerl, V., *Arch. ges. Physiol.*, **231,** 563 (1933).

[8] Hosoya, Y., *Ibid.*, **233,** 57 (1933).

[9] Kühne, W., *Z. Biol.*, **32,** 21 (1895).

[10] Wald, G., *Nature*, **132,** 316 (1933).

[11] Fredericia, L. S., and Holm, E., *Am. J. Physiol.*, **73,** 63 (1925); Tansley, K., *J. Physiol.*, **71,** 442 (1931).

GROUPS INVOLVING THREE AND ONLY THREE SQUARES

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The groups whose operators have no more than three distinct squares were considered in these PROCEEDINGS, **19,** 848–851 (1933), and those whose orders are less than 64 were then completely determined. If the operators of a given group G have three and only three distinct squares, then the operators of the direct product of this G and any abelian group of order 2^m and of type (1, 1, 1, . . .) have the same property. To simplify the statements such direct products will always be excluded in what follows and hence we shall prove that there are eight and only eight distinct groups which satisfy the condition imposed by the heading of the present article. Two of these involve operators of order 3 and are of order 3 and 6, respectively. The remaining six appear in three pairs of the same orders and their orders are 16, 32 and 64, respectively. Since these groups of orders 16 and 32 were fully determined in the article to which we referred the present article is devoted to a proof of the fact that there are two and only two such groups of order 64 and that no such group of a larger order exists.

Since the three operators which are squares include the identity all of these groups are non-abelian because the product of two distinct operators of order 2 which are squares is not a square under G. These two operators are obviously commutative and hence they generate the four groups. This invariant subgroup includes the commutator subgroup of G since the orders of all the operators in the corresponding quotient group divide 2. It is also the central of G because this central contains no operator of order 4 and no operator of order 2 besides those which appear in the given subgroup of order 4 as G is not a direct product. Therefore every operator of G appears in an abelian subgroup of order 16 in view of the following evident theorem: *If the central of a group of order p^m, p being a prime number,*

is of order p^α then every operator of this group which has less than $p^{m-\alpha-1}$ conjugates thereunder appears in an abelian subgroup of order $p^{\alpha+2}$.

These abelian subgroups of order 16 are either of type (1, 1, 1, 1) or of type (2, 1, 1). We shall first prove that there is one and only one G of order 64 which contains the former abelian subgroup and that this G contains only one such subgroup. If G is of this order and contains this abelian subgroup it can contain no larger abelian subgroup of this type since it would otherwise involve more than three distinct squares. Hence this subgroup is invariant under G since it involves its central and therefore its commutator subgroup. Every operator of G which is not found in this subgroup is commutative with exactly eight of its operators since G would otherwise involve more than three operators which are squares under it. Moreover, every operator of this subgroup besides those which appear in the central of G gives rise to the same commutator of order 2 under G and has only two conjugates thereunder.

If every co-set of G with respect to this subgroup of order 16 would contain operators of order 2 then G would involve only two operators which are squares. As this is impossible at least one of these co-sets involves operators of order 4 and these operators have two distinct squares which are different from the commutator of order 2 arising from the given subgroup. It therefore results that all the operators of G which do not appear in this subgroup are of order 4 and have four conjugates under G. These operators of order 4 are contained in six abelian subgroups of type (2, 1, 1) which appear in three distinct pairs such that each pair has in common a subgroup of order 8 and of type (1, 1, 1). The three subgroups of order 32 in G which have the abelian subgroup of order 16 and of type (1, 1, 1, 1) in common are simply isomorphic and G is obtained by extending one of these subgroups by an operator of order 4. Hence there results the following theorem: *There is one and only one group of order* 64 *which contains three and only three operators which are squares and involves the abelian group of order* 16 *and of type* (1, 1, 1, 1).

If there is another group of order 64 which involves three and only three operators which are squares under it then each of its operators appears in an abelian subgroup of order 16 and of type (2, 1, 1). We shall first consider the case when each of the operators of at least one such subgroup has at most two conjugates under G. The commutator subgroup to which the operators of this abelian subgroup give rise under G is then of order 2 and is generated by its operators of order 4 since two commutative operators of order 4 contained in G have a common square. Each of the two co-sets of G with respect to this abelian subgroup of order 16 under which operators of order 4 contained therein are invariant involves eight operators of each of the orders 2 and 4, and these operators of order 4 have the same square as those contained in the given abelian subgroup of order 16. It

is therefore necessary that the remaining co-set with respect to this abelian subgroup of order 16 involves operators of order 4 whose squares include the other operator which is a square under G. Hence all of these 16 operators of order 4 have a common square and G involves 40 operators of order 4 while the G determined above has 48 such operators.

Each of the operators of this G which does not appear in the given abelian subgroup of order 16 has four conjugates under G because two of its operators of order 4 which have different squares cannot be commutative. Since these conditions determine G there results the following theorem: *There is one and only one group of order* 64 *which involves three and only three operators which are squares thereunder and contains an abelian subgroup of order* 16 *and of type* (2, 1, 1) *whose operators have separately at most two conjugates under it.*

If another such G of order 64 would exist each of its operators would appear in an abelian subgroup of order 16 and of type (2, 1, 1) and every one of these abelian subgroups would involve operators which would have four conjugates under G. Moreover, each of these abelian subgroups would involve operators which have only two conjugates under G as well as four invariant operators, for if each of its twelve non-invariant operators would have four conjugates under G the four which are of order 2 would be transformed under G into themselves multiplied by the common square of the eight which are of order 4 by an operator of G which would not transform some one of these eight operators into itself. This is impossible in view of the fact that G involves only three distinct operators which are squares under it. Hence there results the following theorem: *If a group of order* 64 *contains three and only three operators which are squares under it then each of its abelian subgroups of order* 16 *involves operators which have exactly two conjugates under the group.*

To prove that the two groups of order 64 determined above are the only ones which satisfy the prescribed conditions it remains only to prove that such a G necessarily contains an invariant abelian subgroup of order 16 such that each of its 12 non-invariant operators under G has only two conjugates thereunder. To prove this fact we note that the subgroup of order 32 composed of the operators of G which are commutative with one of its non-invariant operators is non-abelian and has a central of order 8 since a central quotient group is always non-cyclic. Hence this subgroup of order 32 contains three and only three abelian subgroups of index 2. The abelian subgroup of order 16 in which a given operator of G which does not appear in this subgroup of order 32 is contained does not involve the central of this subgroup. Hence there is another subgroup of order 32 in G composed of its operators which are commutative with one of its non-invariant operators.

These two subgroups of order 32 have a cross-cut of order 16. It re-

mains to prove that this cross-cut contains their centrals and hence it is the abelian subgroup in question. If this were not the case it would be non-abelian and it and these centrals would generate G. As all of its operators are commutative with every operator of the group generated by these two centrals G would involve two non-abelian subgroups of order 16 which would generate it and would have the property that every operator of each one of these subgroups would be commutative with every operator of the other. This is impossible since all the operators of order 4 in each of these subgroups would have a common square and hence the operators of G could not include exactly three distinct squares thereunder. Hence the given cross-cut is abelian and our theorem is proved.

It remains only to prove that if the order of a group which satisfies the conditions imposed by the heading of the present article exceeds 64 then it is necessarily a direct product. To prove this fact we may first note that every such group involves a non-abelian invariant subgroup of order 16 whose operators have three distinct squares under it. If the order of G would exceed 64 it would contain an operator of order 2 which would be commutative with every operator of this invariant subgroup since its operators are transformed under it according to the four group and they could not be transformed in more than sixteen different ways under G. If the group thus obtained would be contained in a still larger group and would not be a direct product two operators of order 2 which are commutative with every operator of the given subgroup of order 16 would be non-commutative with each other. This is impossible since not all the operators of this subgroup could be commutative with a given operator of order 4 because they have distinct squares.

It should be added that the results stated above as regards the number of groups of order 64 is in disaccord with a theorem noted in the article to which we referred in the first paragraph of this one where three such groups were announced. One of these three is actually a direct product of such a group and an abelian group of type (1, 1, 1, . . .), and hence it should not have been counted. The present article seems to complete by means of more general theorems definitely the determination of all the groups whose operators have no more than three distinct squares. The case when there are four such squares in a group presents much greater difficulties, especially when these four squares constitute the four group. These groups do not satisfy the recently announced theorem relating to the groups whose squares constitute a non-abelian dihedral group (these PROCEEDINGS, **20,** 129 (1934)).

HETEROTOPIC SPINAL CORD GRAFTS IN SALAMANDER EMBRYOS

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Harrison[1] found that any nerve or nerves in whose path a limb was placed was capable of giving rise to intrinsic nerves having a perfectly normal arrangement and that this distribution was determined by the differentiation of structures within the developing extremity. Recent experiments by Detwiler and Carpenter[2] have demonstrated (in Amblystoma) that heterotopic forelimbs innervated by non-brachial nerves may exhibit spontaneous movements and respond reflexly to tactile stimulation. However, they never exhibit swimming or walking reflexes characteristic of a normally innervated limb. They showed also that if one or more brachial nerves contributed to the heterotopic limb, its activities were coördinated with the other extremities. Section of these brachial nerves caused cessation of all coördinated movements. Thus it is evident that normal function of the limb depends upon nervous connections with the normal brachial region of the cord (segments three, four and five).

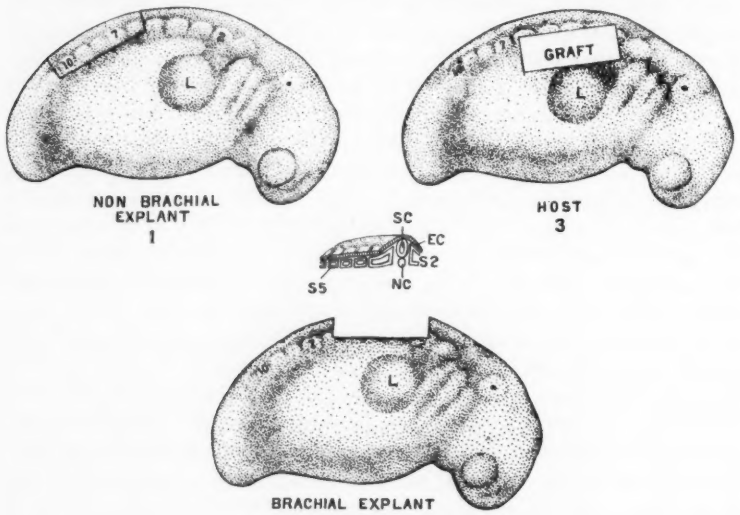
We desired to know whether limbs would exhibit typical appendicular reflexes when supplied by a supernumerary brachial cord. As a means of comparing the potencies of isolated brachial cord (segments 3 to 5, inclusive) with a non-appendicular region (segments 6 to 10), pieces containing each of these regions were grafted to identical positions (Figs. 1 and 2). The operations were done on embryos of Amblystoma punctatum and A. tigrinum in the tail bud stage of development.

The grafts included the spinal cord, the medial portions of adjacent somites, the notochord and the covering ectoderm. They were taken from donor embryos and implanted dorsal to the limb disc of the host embryo (Fig. 3). This position was found previously to be the optimum one for the growth of nerves to the limb (Rogers).[3]

In a preliminary report the author[4] ('32) described the rhythmic walking responses of the limb innervated solely by a supernumerary grafted brachial cord. In these limbs each group of muscles was perfectly timed and coordinated with supporting or opposing groups of muscles. Limbs which functioned entirely on an independent cord and which lacked nervous connections with the central nervous system of the host, were not coordinated in any way with the other extremities. The above observations were based upon eighty experiments of which approximately one-half gave the responses described above.

Thirty animals having spinal segments 6 to 10 grafted dorsal to the limb, and in which all parts of the limb were innervated by the isolated cord, were studied physiologically. In these cases stimulation of the implanted cord or of the independent limb evoked responses from specific groups of muscles, but in no case were such responses similar to normal brachial motions. The motor responses of these limbs were identical with those obtained in heterotopically grafted limbs which became innervated by non-brachial nerves. In no case did we obtain rhythmic coördinated responses.

Serial sections of fifteen cases showed one, two or three nerves from the isolated non-brachial cord innervating the limb. The contraction of vari-



FIGURES 1, 2 AND 3

Figures 1 and 2 (donor embryos) illustrate the region explanted in the two series of experiments.

Figure 3 shows the site of implantation of the explant in the host embryo.

ous groups of muscles upon stimulation of the implant had indicated a normal distribution of nerves within the limb.

Coördination reflexes arising in the brachial cord were found to be independent of the number of nerves which grow into the limb during development, since identical responses were obtained from limbs having one, two, three, four or five nerves from the brachial cord.

In each of these two types of experiments the intrinsic distribution of nerves was typical regardless of segmental number or origin. The only variable in the experiment was the region of the implanted cord which

later innervated the limb, with definite coördination when the limb was innervated by the brachial cord and lack of coördination when innervated by a non-brachial region.

Our physiological findings are in accord with those of Detwiler and Carpenter. They supplement these by demonstrating that the locomotor coördinating mechanism is capable of developing in brachial cords when they are completely isolated from all influences of the central nervous system, previous to the outgrowth of nerves.

[1] Harrison, R. G., *Jour. Exp. Zoöl.*, **4**, 239–281 (1907).
[2] Detwiler, S. R., and Carpenter, R. L., *Jour. Comp. Neur.*, **47**, 427–442 (1929).
[3] Rogers, W. M., *Anat. Rec.*, **58**, 71–91 (1933).
[4] Rogers, W. M., *Anat.*, **52**, supplement 32–33 (1932).